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14. ABSTRACT
Fatty acid synthase (FASN), the enzyme that synthesizes fatty acid in cells, is over-expressed in prostate cancer and a potential therapeutic target. We have identified several novel chemical scaffolds with potential to inhibit FASN. An extensive series of anti-FASN pharmacophores has been synthesized and characterized for their ability to inhibit recombinant FASN, FASN activity in tumor cells, and to kill prostate cancer cell lines. The best inhibitors have increased potency over other FASN inhibitors, including orlistat, the prototype FASN thioesterase inhibitor. This report summarizes the the immense amount of structure-activity-relationships for new compounds we developed and reports on a new click-chemistry approach to derive novel FASN inhibitors.

15. SUBJECT TERMS
fatty acid synthase, thioesterase, inhibitors, drug development

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Introduction

The purpose of this proposal was to develop and optimize chemical scaffolds as potential inhibitors of fatty acid synthase (FASN), specifically the thioesterase (TE) domain. This line of investigation was based on a series of observation by many groups, including ours, that FASN represents a valuable drug target. It isoverexpressed in prostate cancer and appears to be required for tumor cells to survive. Through an iterative scheme of *in silico* design, activity-based screening and structural analyses we identified a series of novel pharmacophores with the ability to inhibit the thioesterase domain of FASN. This proposal had three specific aims. They were 1) To optimize compounds through structure-based design, chemical syntheses and *in vitro* testing, 2) To determine the toxicological and pharmacokinetic properties of the most promising analog(s), and 3) To test the efficacy of the analog(s) in mouse xenograft models of human prostate cancer. Here we summarize the findings by our group during the course of the research proposal.

Body

Our discovery, design and medicinal chemistry efforts have led to the synthesis of more than eighty (80) fully characterized compounds representing six structural classes: 5,6-quinoline-diones, naphthylene-1,4-diones, 1,4-benzoquinones, 1,4-hydroquinones, benzo[*d*]isoxazole-4,7-diones and 1 *H*-indazole-4,7-diones. In addition, numerous precursors, numbering in the hundreds have also been generated. The novel members of these classes are the subject matter of three provisional patent applications. All salient data collected, thus far on these compounds is summarized in Appendix A. We have also developed a new methodology, targeted click-chemistry, for the derivation of novel classes of FASN inhibitors.

I. Pharmacological and *in vitro* data novel compounds

Based on our medicinal chemistry efforts and data collected thus far, the 1 *H*-indazole-4,7-dione scaffold appears to be a flexible template for further optimization. Figure 1 summarizes several compounds we have selected for further optimization, and the data for 86 compounds is summarized in the appendix.

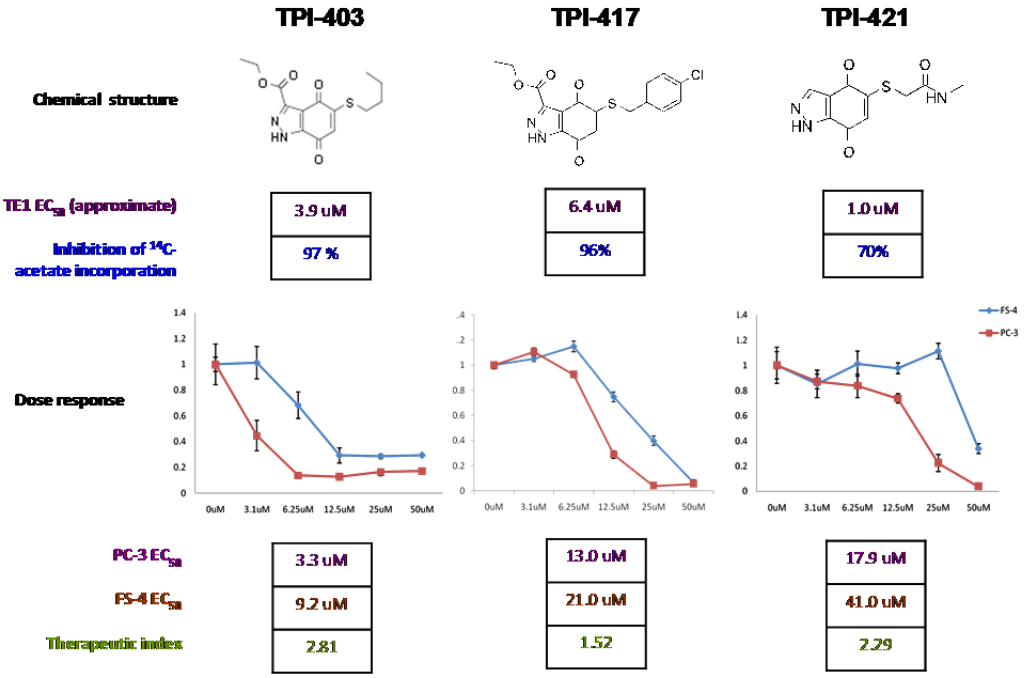


Figure 1- Lead Series Data Summary

Key: PC-3, prostate cancer cells; FS-4, normal fibroblast (control cell line); therapeutic index, {FS-4 EC_{50}}/{ FS-4 EC_{50}}

II. Further optimization strategy for TPI-403, TPI-417 and TPI-421

Further optimization of this series centers on two themes: (1) increasing affinity at TE and (2) increasing solubility in aqueous media. The former goal will also likely lead to a desired increase in therapeutic index of the series (defined here as $EC_{50(normal\ cells)}/EC_{50(cancer\ cells)}$). The structure-activity relationships thus far indicate that a wide variety of substituents are accommodated in Regions A and B of the 1*H*-indazole-4,7-diones scaffold. These regions are depicted in Figure 2.

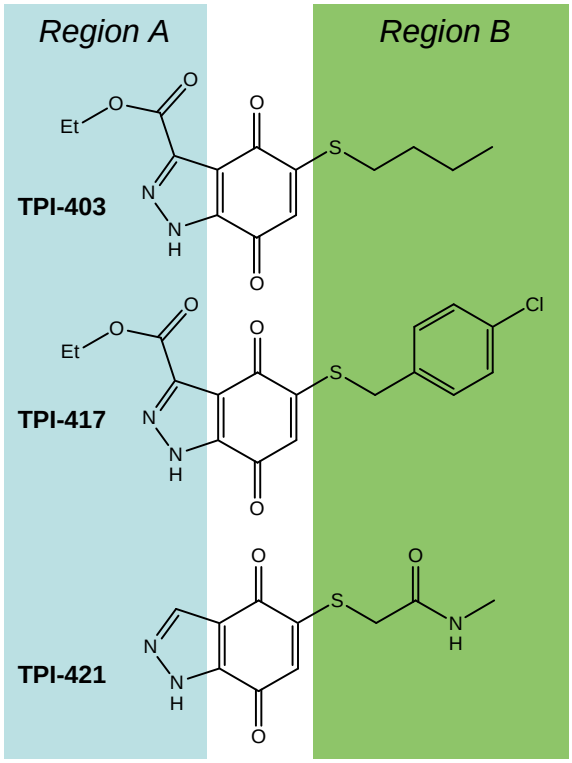


Figure 2- 1*H*-indazole-4,7-dione optimization regions

The further optimization plan for Region A is summarized in Figure 3 and will take advantage of the fact that the 5 position of the 1,4-dihydroquinone intermediate (blue structure, Figure 3) is highly susceptible to nucelophilic attack. In addition, well-established Diels-Alder chemistry will be used to create additional fused ring structures (structures 3d and 3f). Other key targets include: the introduction of various substituents (R1) into the indazole ring of structure 3a; and coupling of various aldehydes and α,β-unsaturated ethers to the 5 position of the quinine under acidic conditions to yield compounds like 3c and 3e.

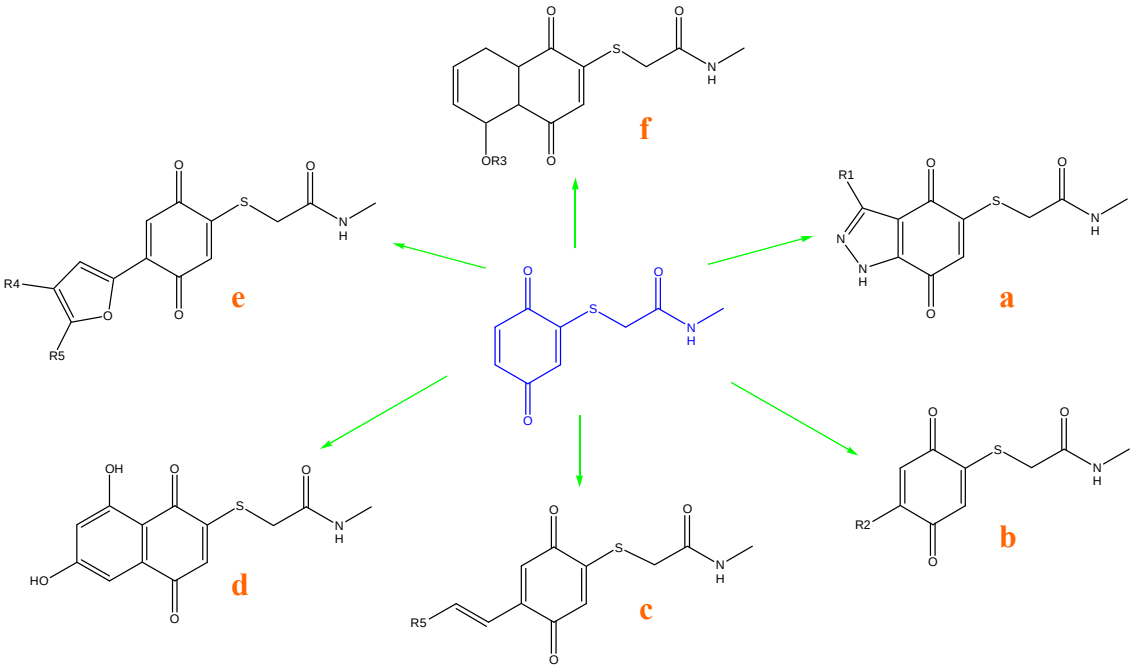


Figure 3- Region A optimization strategy for TPI-403, TPI-417 and TPI-421

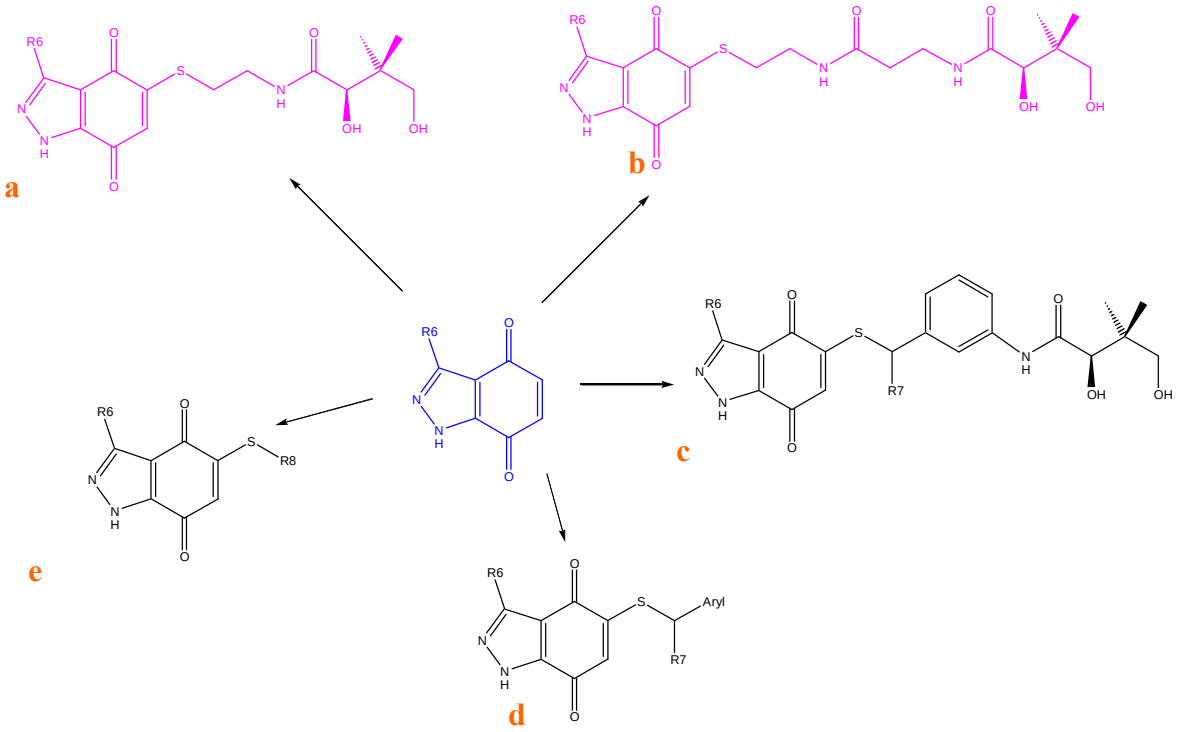


Figure 4- Region B optimization strategy for TPI-403, TPI-417 and TPI-421

The proposed further optimization of Region B, is shown in Figure 4. Here we will take advantage of crystallographic and docking data generated by our laboratories. Together these data demonstrate that substituting a pantetheine moiety onto the 1*H*-indazole-4,7-diones position of the 1*H*-indazole-4,7-dione scaffold (blue structure, Figure 4) would preserve the likely binding mode of the quinone near the catalytic triad of TE while packing the pantetheine channel, which is a unique feature of TE. We surmise that the introduction of a pantetheine moiety in a favorable orientation will not only significantly increase TE affinity and solubility, but will also increase specificity of the series toward the target. Why? Because pantetheine is a cofactor used exclusively for fatty acid synthesis, which is an absolute requirement of epithelial cancer cells and is also known to correlate with tumor aggressiveness. Examples of pantetheine-like target compounds are shown in Figure 4: structures 4a and 4b; structure 4c is an analog of TPI-417 that attempts to preserve the aromatic moiety adjacent to the indazole ring, while introducing key features of pantetheine.

Backup Compounds and Other Findings

III. 5,6-quinoline-diones

Based on our finding that the Nanosyn library compound containing the 5,6-quinoline-dione moiety (TPI-100, see Appendix A for structure) inhibits recombinant FASN TE and cancer cell growth, we pursued development of novel analogs of this 5,6-quinolinedione. Following the synthetic scheme shown in Figure 5 we were able to synthesize 10 5,6-quinoline-dione analogs. While structure-activity relationships indicated a clear trend towards a more optimal biological profile, we turned our attention toward the promising and easily synthesized 1,4-naphthoquinones and 1,4-benzoquinones.The chemistry of the 5,6-quinoline-diones have proven to be challenging due to low yields and lack of 'generalizability'. The overall synthesis up to the hydroxyquinoline stage (structure *d*) is efficient and gives high yields overall, but the critical oxidation step (*d* → *f*) provided only marginal yields and did not work with many of the amines (*e*) of interest.

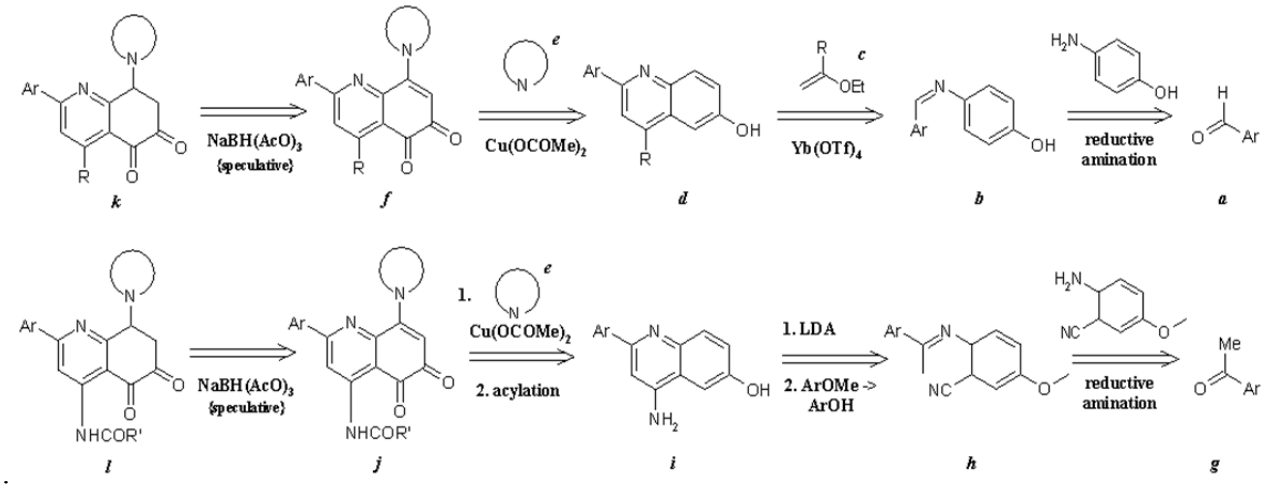
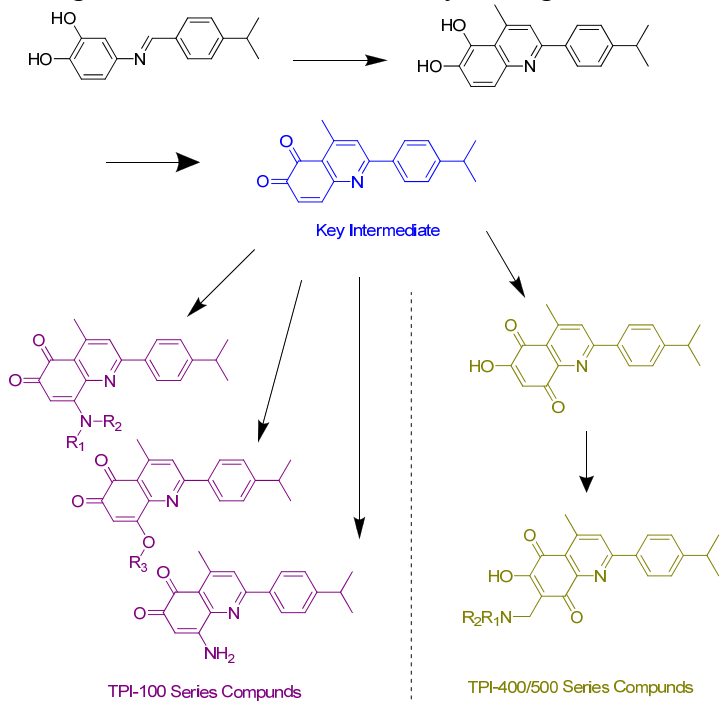


Figure 5- Synthetic strategy for 5,6-quinoline-diones

We have devised an alternative strategy that will hopefully lead to the facile development of 5,6-quinoline-diones as well as additional 1,4-benzoquinones. The overall approach is shown in Figure 6. Using this strategy, we will focus on the further optimization of TPI-107 analogs as a strategy for developing backup compounds.

IV. 1,4-naphthoquinones and 1, 4-benzoquinones As mentioned above, high-throughput screening has identified two 1,4-naphthoquinones, TPI-400 and TPI-500 (see Appendix A for structures) initially analogs of these compounds were developed using the synthesis shown in Figure 7. It is worth noting that TPI-400 proved difficult to synthesize; no attempts were made to synthesize NS-500. One analog (TPI-501) was made as an attempt to reduce the chemical reactivity of the lead compound; unfortunately the pharmacological profile of the compound was very poor. A search of literature compounds and natural products led us to screen numerous 1,4-quinone containing compounds. As a result, we discovered that the natural product juglone (see Appendix A) is a potent inhibitor of TE1. One analog of juglone was synthesized (TPI-404), with the aims of making an analog with less chemical reactivity, but again, the introduction of the N-morpholinyl group was not favored.



During the coarse of this work we also determined that the intellectual property space around the naphthylene-1,4-dione series is rather limited, thus we opted to find alternative scaffolds like the and 1*H*-indazole-4,7-diones described above. We also pursued the development of the closely related benzo[*d*]isoxazole-4,7-diones but we were unable to determine and efficient methodology to construct the fused isoxazole ring, nor were we able to identify an efficient process to oxidize the benzo[*d*]isoxazole-4,7-diol (TPI-401) to yield the desired product.

Figure 6- Future strategy for synthesis of 5,6-quinoline-diones

As seen in the included appendix, we synthesized near ly 90 compounds, not including each of the precurso rs leading to the compounds. Based on multiple criteria including ability to inhibit recombinant enzyme, ability to inhibit fatty acid synthesis in cel ls and tum or cell cytotoxicity, se veral compounds were evaluate *in vivo*. Compounds 414 and 416 (see appendix) were initially chos es. A caveat to both com pounds was that they each had limited solubility, a problem that was common to most of our com pounds. Because both compounds were not very soluble, we chose to perform an MTD (m aximum tolerated dose) study in nude m ice. We tested 5 concentrations from 12.5 to 100 mg/kg delivered by intraperitoneal (IP) injection. The plan was to deliver three doses over three days and observe for t oxicity. All mice either died or had to be terminated following delivery of 414. The 50 and 100 m g/kg mice died after first injection (4/4), the 37.5 and 25 m g/kg (4/4) died after 2 doses and the 12.5 m g/kg (4/4) died after the third dose. Efforts to im prove solubility and specificity are being explored. The cause of toxicity and death has not been determined.

Enhancing discovery horsepower through Click chemistry (CC).

The costs and com plexity of drug d iscovery present a barrier-to -entry for many academic researchers who frequently possess otherwise highl y drug-able targets. In this propos al we address one aspect of this problem: the costly and tim e-consuming process of conve ntional medicinal chemistry. The inspiration behind our efforts to accelerate our FASN Drug Develop m ent Program (FASDDP) comes from the broad fie ld of target-guided synthesis, originally described by Rideout and coworkers (1,2). Target-guided synthesis offers an attractive alternative to traditional lead optimization tech niques. By m aking use of a protein target as a nanoscale reaction vessel, only the building blocks that f it into the confines of the protein binding site(s) can react to form new compounds. In a rece nt extension of this m ethodology known as *in situ* Click chem istry (CC), Rostovtsev and coworkers use the bioorthogonal Hu isgen cycloaddition reaction to identify novel high affinity ligands (3). They and othe r investigators dem onstrated that ve ry high-affinity com pounds can be identified with relatively little effort (4-6). In a CC experiment, a set of alkynes and azides are com bined with target protein in aqueous buffer under am bient conditions. Those a lkynes and azides that bind with an orientation favorable to cycloaddition form new triazole compounds. For exam ple, numerous classes of acetyl-cholinesterase inhibitors have been developed, m any with fe mtomolar binding affinities (7). This body of literature also confirms that the free-solution Huisgen reaction is so slow (by a factor of 10^5) that false-positives are practically nonexistent. F inally, CC is considerably m ore efficient in exploring m olecular diversity than conventional medicinal chemistry approaches. Filling the discovery pipeline with diverse leads is one of the most significant strategies for success in the drug discovery and development process.

We first discovered that the FDA-approved drug Orlis tat can inhibit FASN, sel ectively kill tumor cells and inhibit the growth in prostate tu mor xenograft in m ice (8). Specifically, Orlistat inhibits the thioesterase (TE) domain of FASN, the term inal step of fatty acid synthesi s. We subsequently so lved the first crystal structure of FASN-TE bound to Orlistat (9). This structure revealed that FASN-TE contains three distinct binding pockets. The specificity or hydrophobic channel binds the growing fatty acid chain and guides substrate specificity of the enzyme. The short-chain pock et contains the active site serin e of the enzyme. Lastly, th e pantetheine channel inte racts with the acylated acyl-carrier protein of FASN. These resu lts, combined with previous studies, highlight the broad potential of FASN as a therapeutic target and suggest multiple strategies to block enzyme activity. Moreover, one could e nvision that the three unique binding pockets provide m ultiple environments to accommodate Click fragments in novel conformations.

Although Orlistat is an FDA-approved drug, it has several shortcomings that limit its potential as an anti-cancer therapeutic. The driving factor is that Orlistat does not reach systemic circulation. Rather it is active in the gut and that which reaches circulation is rapidly inactivated. Based on these facts, a combined activity- and structure-based chemical library screening strategy was devised to identify novel chemical scaffolds with

potential to inhibit FASN-TE (**Fig. 7, left column**). This strategy follows the traditional drug discovery paradigm. Using a library of 8,800 compounds, a subset of 221 hits was identified as potential FASN inhibitors. In an effort to increase the novelty and diversity of the chemical scaffolds with FASN-inhibitory potential, a CC approach was devised (**Fig. 7, right column**). We took advantage of the existing data in hand and generated a library of 19 alkynes (A fragments) and 11 azides (Z fragments). Each A and Z fragment was selected based their enriched occurrence in the initial pool of 221 FASN leads. The A-Z pairings represent a focused library of 209 combinations. In addition to the presence in the initial screen, the 19 A fragment and 11 Z fragments were also vetted against patent art for "uniqueness" as anti-cancer compounds. To generate the Click leads, each A-Z pair was incubated overnight with recombinant FASN-TE. If an A and Z fragment bind within the FASN-TE active site with the correct special orientation, the azide and alkyne moieties will spontaneously react to form the Click compound. After the overnight reaction at room temperature, the reaction was stopped and enzyme was removed by precipitation. The formation of Click compounds was determined by screening for the unique mass signature of each theoretical compound by mass spectrometry. The experiment yielded 23 unique Click compounds, a high proportion consistent with the focused nature and design of the A/Z fragment library. These are represented in the "*Completed*" portion of **Figure 7**. From these 23, we plan to synthesize 6 compounds based on: 1) availability of reagents, 2) ease of predicted synthetic strategy, and 3) prediction of FASN inhibitory potential. The A fragments are highlighted in green and the Z fragments are highlighted in blue. This is represented in the "*Proposed*" portion of **Figure 7**. The "*Future*" portion of the figure shows a possible core modification that may be pursued. We anticipate that core modification will be necessary to optimize the chemical functional groups further and to define a core that is clear of any potential patent space or intellectual property issues

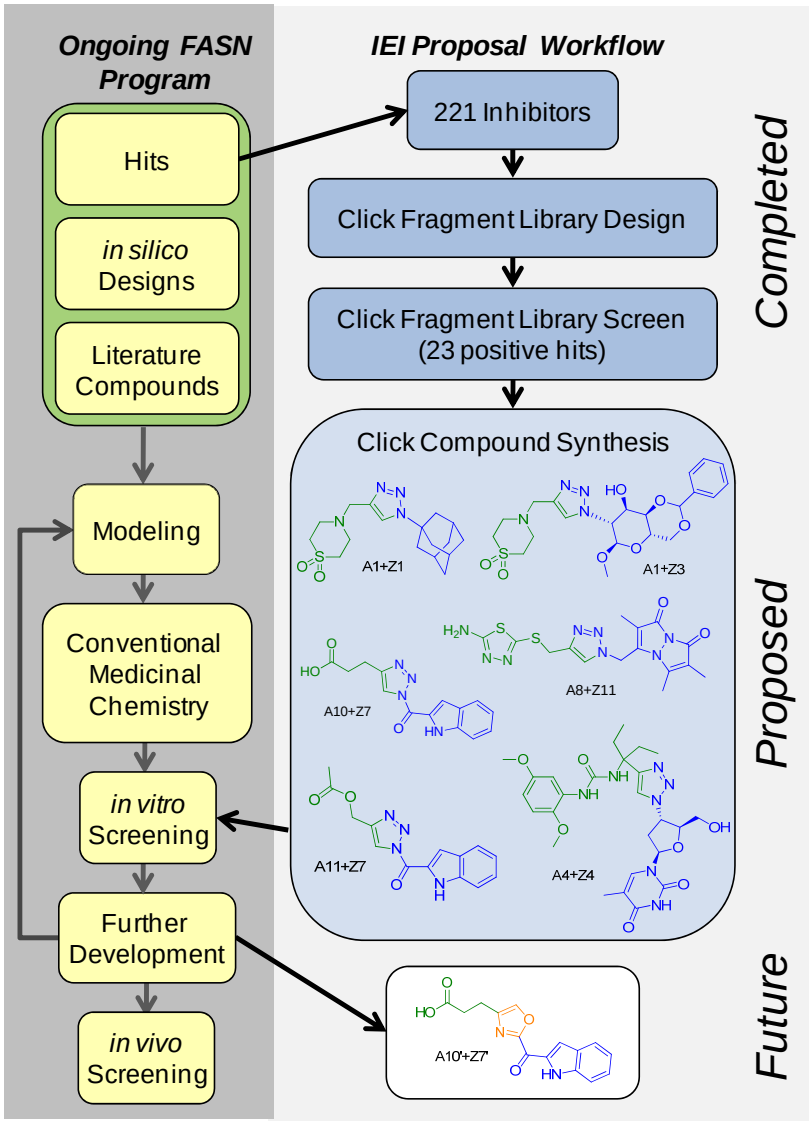


Figure 7. Proposed Click chemistry workflow to develop FASN inhibitors. The left column represents a traditional ongoing FASN drug discovery program. The right column is the proposed work flow for the proposed Click chemistry strategy. *Completed*: The generation of 23 potential Click leads against FASN has been performed. *Proposed*: The 6 Click-derived compounds that will be synthesized and screened for the ability to inhibit FASN. *Future*: A modified version of the A10-Z7 lead where the triazole ring is replaced with a different moiety.

Synthesis of Click leads. We have identified the leads and now are planning the synthesis phase of the plan. From the initial 23 Click hits, 6 azide and alkyne fragment combinations that produce triazole product, will be re-synthesized for structural verification and further screening. Briefly, copper(I)-catalyzed cycloaddition will be used to regenerate the triazole Click Leads in the regioselective, *anti* configuration, according to the method of Tournøe (10). In this procedure, one equivalent each of alkyne and azide are stirred

at room temperature in THF:H_2O (1:1) along with copper iodide (2 equi valents) and diisopropylethylamine (50 equivalents). After 16 hours the de sired product forms in high yields. C onfirmation of chemical structure and geometric isomerism will be d etermined by ^{1}H, ^{13}C and 2D NOE NMR techniques. For exam ple, the lone triazole proton of 1,4-substitu ted [1,2,3]-triazoles will be shifted cons iderably downfield compared to 1,5-substituted [1,2,3]-triazoles (11). This resonance sh ift will provide supporting evidence that the copper(I)-catalyzed 1,3-dipolar cycloadditi on only gives the 1,4-regioisom er (*anti* configuration). Furtherm ore, NOE effects will be observed between the triazole proton and the *N*-substituted alkyl group next to it (see **Fig. 2**). Where needed, purification of the Click Leads will be conducted using either preparative HPLC or open-column flash chromatography.

There a several significant points to be made about this strategy. First, to our knowledge, this strategy is unique for the identification of FASN inhibitors. Second, the strategy took advantage of chemotypes with known FASN-TE inhibitory capacity, so there is extra optimization. Lastly, all of the compounds put into the screen were pre-vetted for their novelty in terms of intellectual property. Should useful compounds be derived, they will certainly have IP potential as well as clinical utility.

Key Research Accomplishments:

- Synthesis and characterization of more than 80 novel FASN inhibitor scaffolds. (see Appendix)
- Optimization of FASN inhibitors of novel chemotypes
- Development of new synthetic strategies and avenues to generate FASN inhibitors

Reportable Outcomes:

Manuscripts

1. DeFord-Watts, L.M., Mintz, A. and **Kridel, S.J**., The Potential of ^{11}C-acetate PET for Monitoring the Fatty Acid Synthesis Pathway in Tumors (2010) *Current Pharmaceutical Biotechnology, In press*
2. Odens, H.H., **Kridel, S.J**., Lowther, W.T., Watts, L.M., Filipponi, L.E., and Schmitt, J.D., Inhibition of the Thioesterase Activity of Human Fatty Acid Synthase by 1,4-Quinones. *Submitted*
3. **Kridel, S.J.**, Johnson, L., Wheeler, F., Filipponi, L.E., Odens, H.H., Schmitt, J.D., and Lowther, W.T., Structure Activity Relationships of Novel napht hoquinones that target the Fatty Acid Synthase Thioesterase Domain. *In preparation*.
4. Scott, K.E.N., and **Kridel, S.J**., Fueling the fat cravings of a tumor cell. *In preparation*.

Funding received, based on this award

1. SPARK Grant (Innovation and Entrepreneurship Initiative) 10/01/11-9/30/12
   Kridel (PI)
   Development of Novel Fatty Acid Synthase Inhibitors through Targeted Click Chemistry
   The goal of this project is to use Click-chemistry to evolve new FASN inhibitors from enriched fatty acid synthase fragments that have previously been identified.

   This intramural grant is an extension of the work from this LCT award and will provided a small pool of support to pursue some novel click-chemistry methodologies to derive novel FASN inhibitors from our previously determine pool of validated FASN inhibitors.

2. R01 CA161503 NIH/NCI 07/01/12-04/31/17
   Kridel, PI (4.2 months)

NAD^+ metabolism in prostate cancer
The goal of this project is to determine the role of NAD+ metabolism in prostate cancer. Specific emphasis will be placed on understanding the integration of Nampt, the sirtuins and CD38 in the regulation of lipid metabolism and survival of prostate tumor cells.

This NCI funded award is not directly related to the DOD sponsored LCT award, but some of the work from the LCT award resulted in preliminary data that helped the application receive a fundable score.

Transition of trainees to faculty positions

1. Herman H. Odens, PhD: Dr. Odens was hired as a postdoctoral fellow to perform synthetic and medicinal chemistry and was promoted to Instructor in the Department of Biochemistry. Dr. Odens was recently hired as an Associate Professor in the Department of Chemistry at Southern Adventist University in Collegedale, Tennessee.

2. Laura M. Watts, PhD: Dr. Watts was hired as a postdoctoral fellow to characterize novel fatty acid synthase inhibitors in several cancer models. At the conclusion of her training, Dr. Watts was hired as Assistant Professor in the Department of Biology at Salem College in Winston-Salem, North Carolina.

Conclusion

As detailed in the body of this final report, we have synthesized a significant library of potential FASN inhibitors and expanded the potential repertoire of FASN inhibitors through a novel click-chemistry approach. These results highlight the significant effort that has been put forth as well as the hurdles that have been overcome. The implications of our finding are significant on multiple levels. First, the resulting data provides significant structure-activity-relationship (SAR) information around the active site of the TE domain of FASN. Because the FASN-TE domain of FASN is increasingly recognized as a potential therapeutic target in cancer, this information could make significant contribution to the development of FASN TE inhibitors, by our group or another. Second, the chemical scaffolds could provide the building blocks from which imaging probes could be derived. Third, and perhaps most important, is that we have identified a novel mechanism by which to identify new FASN inhibitors, that is click chemistry. In using the FASN-TE active site as the reaction vessel we have demonstrated the possibility that highly optimized inhibitors could be generated using that natural chemistry of the active site. The continued development of the click-derived compounds may elucidate not only new potential inhibitors, but also strategies to further optimize them.

It is clear from our studies, as well as those from others, that the development of FASN inhibitors for translation into the clinic will not be an easy task. The TE domain, while attractive as a target, may be hampered by it association with the serine hydrolase family of proteins. The expansiveness of this family may increase potential for reduced specificity.

So what does this body of knowledge contribute? Several academic laboratories along with large and small pharma companies have or are currently developing inhibitors against FASN. For that matter, targeting metabolic enzymes is becoming a more attractive strategy in many types of cancers. The work presented in this report highlight design and optimization of novel FASN inhibitors. The results of this work will contribute to the development of FAS inhibitors and provide an avenue toward the translation of FAS inhibitors into the clinic for potential use in treating men with prostate cancer. It will do so through the identification of new chemical scaffolds that can target FASN and through the description of new click chemistry methodologies that utilize FASN as a chemical reaction vessel.

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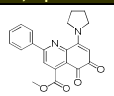
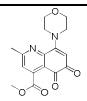
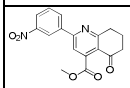
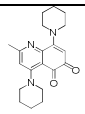
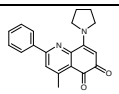
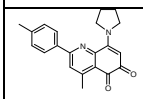
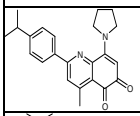
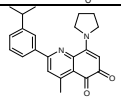
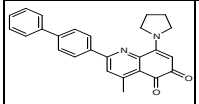
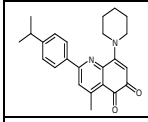
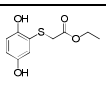
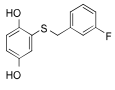
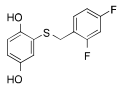
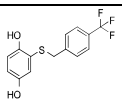
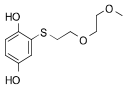
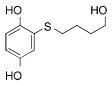
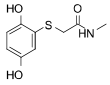
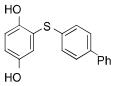
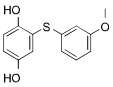
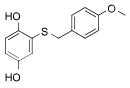
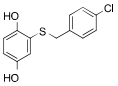
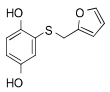
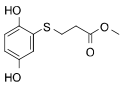
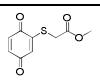
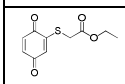
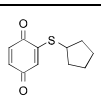
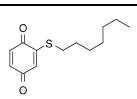
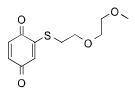
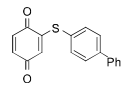
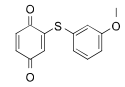
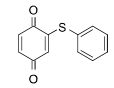
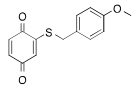
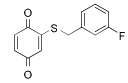
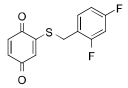
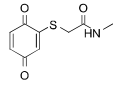
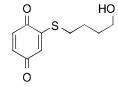
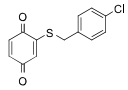
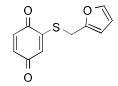
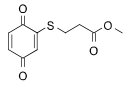
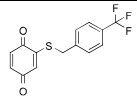
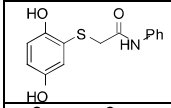
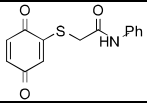
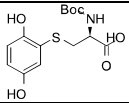
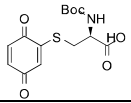
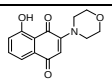
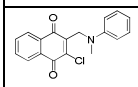
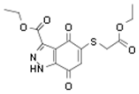
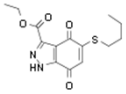
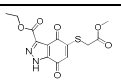
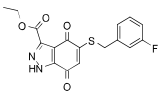
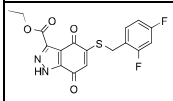
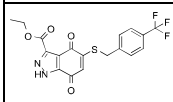
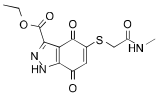
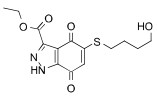
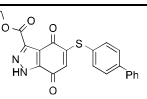
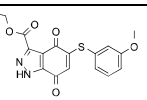
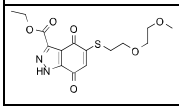
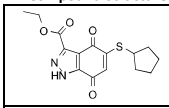
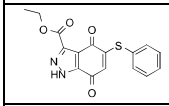
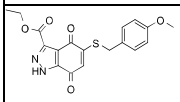
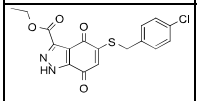
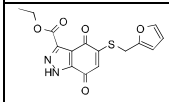
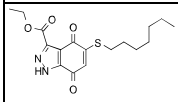
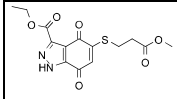
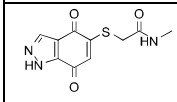
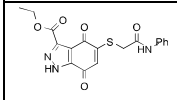
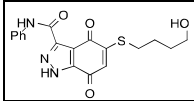
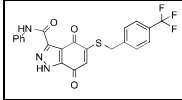
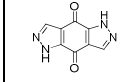
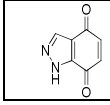
Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
5,6-quinolinediones										
	TPI-00100-00-A (NS-1456)	20.21	22.87	NA	NA	89.3	2.36	2.93	4.2	**1.78**
	TPI-00101-00-A	2.64	8.83	NA	NA	ND	6.4	6.36	ND	ND
	TPI-00102-00-A	17.27	14.45	NA	NA	ND	ND	ND	ND	ND
	TPI-00103-00-A	1.59	11.39	NA	NA	ND	>10	>10	ND	ND
	TPI-00104-00-A	0.00	12.04	NA	NA	ND	ND	ND	ND	ND
	TPI-00105-00-A	9.20	17.59	NA	NA	34	> 25	> 25	ND	ND
	TPI-00106-00-A	37.72	42.72	NA	NA	31	> 25	20.1	16.7	ND
	TPI-00107-00-A	51.68	54.24	11.59	13.78	49	18.2	14.33	9.4	0.52
	TPI-00108-00-A	21.26	26.36	NA	NA	23	> 25	> 25	ND	ND
	TPI-00109-00-A	30.25	26.80	NA	NA	7	> 25 uM	> 25 uM	ND	ND
	TPI-00110-00-A	28.01	46.00	NA	NA	ND	ND	ND	ND	ND
1,4-benzoquinones & 1,4-hydroquinones										
	TPI-00600-00-A	85.94	96.72	7.27	NA	56.3	29.2	>50	9.5	0.33
	TPI-00601-00-A	94.42	96.05	7.05	NA	65.7	32.2	>50	20.8	0.65
	TPI-00605-00-A	30.62	46.98	NA	NA	26.7	ND	ND	ND	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
	TPI-00606-00-A	10.10	14.34	NA	NA	20.3	ND	ND	ND	ND
	TPI-00607-00-A	45.23	19.65	NA	NA	34.3	ND	ND	ND	ND
	TPI-00608-00-A	35.37	93.22	NA	NA	86.2	22.4	>50	>50	ND
	TPI-00609-00-A	78.46	96.16	NA	NA	89.6	23	>50	32.4	**1.41**
	TPI-00611-00-A	31.33	8.42	NA	NA	24.5	ND	ND	ND	ND
	TPI-00612-00-A	0.00	47.84	NA	NA	6.25	ND	ND	ND	ND
	TPI-00613-00-A	13.21	70.02	NA	NA	18.9	ND	ND	ND	ND
	TPI-00614-00-A	37.05	89.56	NA	NA	35.2	ND	ND	ND	ND
	TPI-00615-00-A	18.19	NA	NA	NA	44.6	ND	ND	ND	ND
	TPI-00616-00-A	95.72	95.19	NA	NA	46.7	ND	ND	ND	ND
	TPI-00618-00-A	22.84	90.89	NA	NA	63.6	ND	ND	ND	ND
	TPI-00619-00-A	18.68	85.27	NA	NA	27.7	ND	ND	ND	ND
	TPI-00602-00-A	100.00	100.00	1.19	NA	33.3	37.3	>50	40	**1.07**
	TPI-00603-00-A	100.00	100.00	1.51	NA	16	>50	>50	>50	ND
	TPI-00604-00-A	100.00	100.00	NA	NA	ND	ND	ND	ND	ND
	TPI-00610-00-A	98.76	99.33	6.45	0.85	11.2	ND	ND	ND	ND
	TPI-00617-00-A	99.24	98.94	6.24	0.36	45	ND	ND	ND	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	recombinant thioesterase IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
	TPI-00620-00-A	98.13	98.88	1.70	0.12	91.4	20.8	ND	41.7	**2.00**
	TPI-00621-00-A	100.00	100.00	1.06	0.17	23.4	ND	ND	ND	ND
	TPI-00622-00-A	100.00	100.00	0.70	0.18	11.4	ND	ND	ND	ND
	TPI-00623-00-A	100.00	100.00	1.16	0.20	9.25	ND	ND	ND	ND
	TPI-00624-00-A	100.00	100.00	1.34	0.42	57.7	33	ND	20	0.61
	TPI-00625-00-A	100.00	100.00	1.10	0.25	64.3	22	ND	40	**1.82**
	TPI-00626-00-A	100.00	99.70	1.09	0.40	42.2	ND	ND	ND	ND
	TPI-00627-00-A	100.00	99.49	1.08	0.10	66.4	19	ND	37.5	**1.97**
	TPI-00628-00-A	100.00	97.81	1.16	0.23	53.7	>50	ND	>50	ND
	TPI-00629-00-A	100.00	100.00	1.08	0.24	50	ND	ND	ND	ND
	TPI-00630-00-A	100.00	100.00	1.55	0.34	61.85	ND	ND	ND	ND
	TPI-00631-00-A	100.00	100.00	1.44	0.11	91.9	>50	ND	42	ND
	TPI-00632-00-A	100.00	100.00	1.35	0.36	49.05	ND	ND	ND	ND
	TPI-00633-00-A	76.13	98.99	NA	NA	94.2	24.3	ND	38	**1.56**
	TPI-00634-00-A	100.00	100.00	NA	NA	69.7	24	ND	38	**1.58**
	TPI-00635-00-A	28.21	9.88	NA	NA	0	>50	ND	>50	ND
	TPI-00636-00-A	81.39	100.00	NA	NA	0	>50	ND	>50	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
naphthylene-1,4-diones, benzo[d]isoxazole-4,7-diones & 1H-indazole-4,7-diones										
	juglone	100.00	100.00	0.09	0.07	95.6	6.4	8.7	5.49	0.86
	TPI-00404-00-A	29.00	43.00	NA	NA	ND	ND	ND	ND	ND
	TPI-00400-00-A (NS-4390)	22.48	61.70	NA	NA	ND	29	>25	25.29	0.87
	TPI-00500-01-C (NS-4393)	100.00	100.00	1.08	0.41		18.75	19.2	ND	ND
	TPI-00501-01-A	44.28	34.75	NA	NA		ND	ND	ND	ND
	TPI-00401-00-A	40.00	55.88	NA	NA	29.1	33	>50	ND	ND
	TPI-00402-00-A	66.63	95.73	2.35	0.56	90.4	18.75	40	20.9	**1.11**
	TPI-00403-00-A	69.35	79.27	3.90	2.42	97 (IC_{50} = 6.75 μM)	3.25	15.6	9.15	**2.82**
	TPI-00405-00-A	69.52	96.82	2.78	0.41	ND	ND	ND	ND	ND
	TPI-00406-00-A	73.64	96.01	NA	NA	37.7	ND	ND	ND	ND
	TPI-00407-00-A	65.96	94.89	NA	NA	37.8	ND	ND	ND	ND
	TPI-00408-00-A	67.87	96.18	NA	NA	89.9	18.5	ND	22.5	**1.22**
	TPI-00409-00-A	53.50	92.17	NA	NA	0	ND	ND	ND	ND
	TPI-00410-00-A	59.61	91.67	NA	NA	1.85	ND	ND	ND	ND
	TPI-00411-00-A	70.51	97.43	NA	NA	0.75	ND	ND	ND	ND
	TPI-00412-00-A	81.63	97.98	NA	NA	9.95	ND	ND	ND	ND
	TPI-00413-00-A	50.23	94.78	NA	NA	20.2	ND	ND	ND	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
	TPI-00414-00-A	52.21	86.40	NA	NA	92.4	25	ND	33	**1.32**
	TPI-00415-00-A	79.69	97.46	ND	ND	7.05	ND	ND	ND	ND
	TPI-00416-00-A	64.31	96.49	ND	ND	89.9	25.8	ND	33	**1.28**
	TPI-00417-00-A	74.81	97.85	ND	ND	96.1	13.8	ND	21	**1.52**
	TPI-00418-00-A	65.54	95.92	ND	ND	92.2	31.7	ND	37	**1.17**
	TPI-00419-00-A	66.19	95.79	ND	ND	67.95	32	ND	50	**1.56**
	TPI-00420-00-A	61.96	95.35	ND	ND	68.5	37.5	ND	42	**1.12**
	TPI-00421-00-A	100.00	100.00	1.02	ND	70	17.9	ND	41	**2.29**
	TPI-00422-00-A	22.92	86.76	NA	NA	0	48	ND	>50	ND
	TPI-00423-00-A	79.75	97.99	NA	NA	87.25	20	ND	25	**1.25**
	TPI-00424-00-A	92.59	100.00	NA	NA	0	27	ND	50	**1.85**
	TPI-00425-00-A	0.00	73.61	NA	NA	11.1	ND	ND	ND	ND
	TPI-00426-00-A	48.37	79.24	NA	NA	30.1	ND	ND	ND	ND